Hynobius-takedai (Amphibia, Urodela), a new species of salamander from Japan

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Hynobius takedai (Amphibia, Urodela), a New Species of Salamander from Japan

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ABSTRACT — A new species of salamander, *Hynobius takedai*, is described from the lowland of Ishikawa Prefecture, on the Japan Sea side of the Chubu District, Central Japan. It belongs to the *lichenatus* group of *Hynobius*, and is characterized by the intermediate adult morphology between *H. lichenatus* and *H. abei*, large clutch size, absence of striations on the egg sac envelope, and unique electrophoretic pattern of serum proteins. The new species is considered most closely related to *H. lichenatus* of northeastern Japan.

Salamanders of the genus *Hynobius* are roughly divided into two types, lowland still-water one and mountain stream one, chiefly by the breeding habit and the shape of adult tail [1]. The members of the former type are further split into the *nebulosus* and *lichenatus* groups [2]. With the exception of *Hynobius nigrescens*, which has peculiar egg sac and adult morphology, all the known forms of these two groups from Honshu, the mainland of Japan, particularly preserved specimens, are very difficult to identify because of their remarkable morphological uniformity.

Although members of the *nebulosus* and *lichenatus* groups having transparent "banana" shaped egg sacs are widely distributed in Honshu, they have been reported to be absent in some areas. The Hokuriku District had been among such areas until Mr. Toshio Takeda found a salamander with the characteristics mentioned above from near Koshijino Elementary School, Chijimachi, Hakui-shi, Ishikawa Prefecture on April 10, 1971.

Subsequent surveys made by one of us (KM) proved that the salamander widely occurred in the lowland of Ishikawa Prefecture and had morphological and reproductive characteristics similar to those of *H. abei*. He then identified it with the latter species [3, 4]. *Hynobius abei*, a member of the *lichenatus* group, has been recorded only from a confined area of the Kinki District and is well known for its unique morphology and early breeding season [5]. Miyazaki's [3, 4] identification was chiefly based on the peculiar secondary sexual characters developed in males of the salamander from Ishikawa Prefecture. However, ample specimens of *H. abei* and *H. lichenatus* for comparison were not available for him at that time.

In recent years, we have been intensively studying the salamander in question, and have reached the taxonomic conclusion that the salamander is closer to *H. lichenatus* of northeastern Japan than to *abei*, but is still distinguished at the species level from the former by several characteristics which are considered taxonomically important.

Hynobius takedai
M. Matsui et Miyazaki
sp. nov.

[Japanese name: Hokuriku-sansyouo]

Figure 1

Hynobius abei: Miyazaki, 1977 [3], p. 46.— Miyazaki, 1978 [4], p. 21, figs. 1–2.

Holotype — National Science Museum, Tokyo

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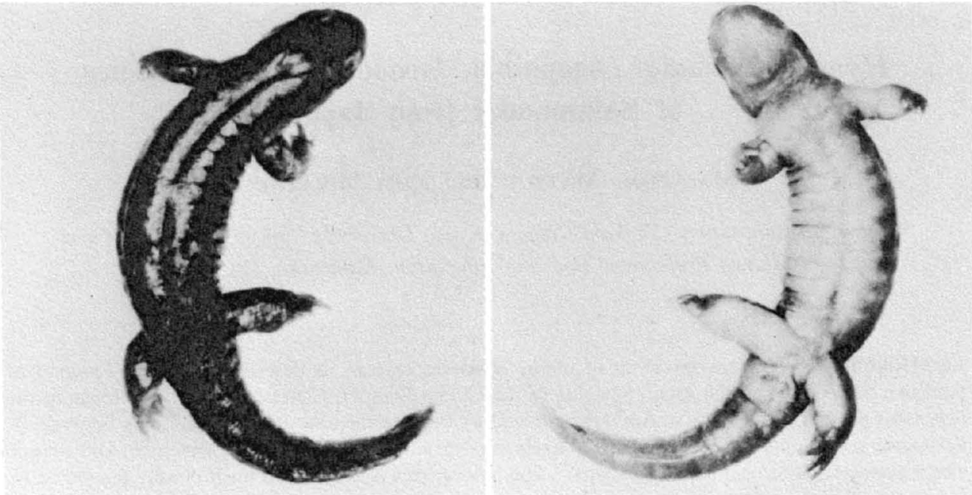

FIG. 1. Dorsal and ventral views of holotype of *Hynobius takedai* (NSMT–H–03990).

(NSMT)–H–03990, an adult male, collected in a small pool, Chiji-machi, Hakui-shi, Ishikawa Prefecture, on February 5, 1983, by T. Takeda.

Paratypes — NSMT–H–03991, one female, from the same locality as the holotype, March 4, 1977, by K. Miyazaki; Osaka Museum of Natural History (OMNH) Am 7693, one male, from the same locality as the holotype, April 16, 1978, by K. Miyazaki; NSMT–H–03992, OMNH Am 7694, 2 males, from Shiotsu, Nakajima-machi, February 25, 1979, by K. Miyazaki; OMNH Am 7695, one male from Shiotsu, Nakajima-machi, February 24, 1980, by K. Miyazaki; NSMT–H–03993, one female, from Sohama-machi, Nanao-shi, April 8, 1983, by J. Yamamoto; OMNH Am 7696, one female, from Hosoguchi-machi, Nanao-shi, August 5, 1978, by M. Sakai; NSMT–H–03994–03997, OMNH Am 7697–7701, 9 males, NSMT–H–03998–03999, OMNH Am 7702, 3 females, Akakura-yama, Tatsuruhama-machi, February 6, 1983 by T. Takeda, Y. Akita, K. Miyazaki and M. Matsui.

Diagnosis — A member of the *Hynobius lichenatus* group [2]: breeding in still-water; adapted to cool climate; differing from others in the following combination of morphological characters: from *H. nigrescens* and *H. sadoensis* in smaller body size, less depressed head, shorter tail and limbs, and transparent egg sacs; from *H. lichenatus* in having shorter head and limbs, adpressed limbs usually separated, in having shorter and higher tail, more uniformly dark color of back, usually without yellowish speckles, larger clutch size and in lacking evident longitudinal striations on the envelope of egg sacs; from *H. abei* in having shorter and narrower head, longer limbs, less keeled lower tail with more pointed tip, in the presence of dark spots on the back of some females, and in lacking striations on the envelope of egg sacs.

Description and variation — Morphometric data on 24 males and 7 females are summarized in Table 1 with those on the allied species, *H. lichenatus* and *H. abei* (all measurements were taken on preserved specimens). Head moderately depressed, distinctly longer (% length/SVL 22.0–25.9) than broad (% width/SVL 17.8–21.3). Males with relatively great head both in length (HL) and width (HW) than in females, when each dimension converted to percentage ratio to snout-vent length (SVL) (Mann-Whitney's U-test: $z_{7,24}=3.52$, $p<0.001$, and $z_{7,23}=2.67$, $p<0.01$, respectively; Table 2). Contrariwise, females with relatively long trunk ($z_{7,24}=3.50$, $p<0.001$). Number of costal grooves 12 to 13 (Table 3), modal number being 12 in both sexes. Limbs short and stout, and when adpressed, they overlap at most one costal

TABLE 1. Comparisons of measurements (means±2SE, followed by ranges in parenthesis, in mm) in the three forms of the *Hynobius lichenatus* group

Form	Sex	N	SVL	HL	TRL	TAL	HW	MTAH
H. takedai	M	24	57.2±2.0 (45.6–66.8)	13.8±0.4 (10.8–15.9)	43.4±1.6 (34.8–51.2)	44.0±2.4 (30.2–51.3)	11.4±0.4 (9.9–12.8)	7.6±0.5 (5.3–11.6)
	F	7	57.8±2.0 (53.5–60.0)	13.2±0.3 (12.6–13.8)	44.5±1.5 (40.9–46.8)	38.1±2.9 (32.7–44.8)	10.9±0.5 (9.8–11.8)	6.0±0.6 (5.2–6.8)
H. lichenatus	M	37	58.5±1.9 (42.6–68.5)	14.7±0.5 (10.9–17.3)	43.8±1.5 (31.7–52.3)	49.7±2.3 (32.3–58.8)	11.3±0.5 (8.8–13.8)	7.1±0.5 (4.2–9.2)
	F	2	53.8 (53.4–54.1)	13.6 (13.2–13.9)	40.2 (40.2)	38.5 (36.1–40.8)	10.5 (10.1–10.9)	6.2 (5.7–6.6)
H. abei	M	24	57.8±2.1 (48.5–68.0)	15.0±0.4 (13.2–16.9)	42.8±1.8 (37.2–51.2)	42.9±2.2 (34.8–52.8)	12.3±0.4 (10.8–14.2)	10.0±0.8 (7.3–14.3)
	F	34	55.0±1.2 (45.3–64.4)	13.9±0.3 (12.2–15.5)	41.2±1.0 (32.6–48.9)	35.2±1.2 (28.8–41.8)	11.0±0.2 (9.9–12.1)	7.0±0.3 (56.–8.8)

SVL=snout-vent length; HL=head length; TRL=trunk length; TAL=tail length; HW=head width; MTAH=maximum tail height.

TABLE 2. Comparison of percentage ratio of each character dimension to SVL (medians, followed by ranges in parenthesis) in the three forms of the *Hynobius lichenatus* group

Form	Sex	N	HL	TRL	TAL	HW	MTAH
H. takedai	M	24	24.2 (22.9–25.9)	75.9 (74.1–77.1)	78.3 (56.8–88.9)	19.7 (17.8–21.3)	13.0 (11.4–17.4)
	F	7	23.0 (22.0–23.7)	77.0 (76.3–78.0)	64.7 (61.1–78.0)	18.7 (18.3–19.7)	10.3 (8.9–12.1)
H. lichenatus	M	37	25.3 (23.3–26.8)	74.7 (73.2–76.7)	85.1 (69.4–102.0)	19.2 (15.9–22.0)	12.1 (8.7–15.2)
	F	2	25.2 (24.7–25.7)	74.8 (73.4–75.3)	71.6 (66.7–76.4)	19.5 (18.9–20.1)	11.5 (10.7–12.2)
H. abei	M	24	26.1 (23.4–28.2)	74.0 (71.8–76.6)	73.7 (59.2–97.9)	21.5 (17.9–23.1)	17.1 (12.2–21.7)
	F	34	25.4 (23.5–28.0)	74.6 (72.0–76.5)	64.5 (50.9–74.3)	20.1 (18.0–22.3)	12.8 (10.5–15.1)

Abbreviations as in Table 1.

fold and are usually separated by up to 2.5 folds. Separation is greater in females (median=2 folds) than in males (median=0.5 folds; Table 3, $\chi^2=9.34$, dF=1, $0.001<p<0.01$). Tail vertically oval at base, gradually flattening to middle portion, and increasingly flattened to tip, which is obtusely pointed. Tail moderately keeled above and below, the upper keel originating at the position opposite to the posterior end of vent, developing into tail fin in breeding males. Tip of tail fin pointed. Statistically significant sexual dimorphism in tail shape: males with longer ($t=2.54$, dF=27, $0.01<p<0.02$) and higher ($t=3.04$, dF=26, $0.005<p<0.01$) tail than in females. Fifth toe sometimes rudimentary, but usually present: in observed samples, the 5th toe was absent only on one side of paired hindlimbs in a few males, and 82.6% of males and all of females possessed it on both hindlimbs. Vomerine teeth in two small, obliquely arched series, nearly touching at midline, and forming shallow "U" or "V" shape. Combined series distinctly wider than long (VTW/VTL 1.38–2.66). No sexual difference in the number of vomerine teeth (Table 4, $t=0.55$, dF=28, $p>0.5$). Width of vomerine teeth series (VTW) larger in males than in females ($t=2.97$, dF=19, $0.005<p<0.01$, Table 4), but the length (VTL) does not differ ($t=0.07$, dF=19, $p>0.8$). Shape of vomerine teeth series not sexually different as shown by

TABLE 3. Variation in the number of costal grooves and of costal folds between adpressed limbs in the three forms of the *Hynobius lichenatus* group

Form	Sex	Number of costal grooves			Overlap of adpressed limbs shown by number of costal folds										
		11	12	13	−3	−2.5	−2	−1.5	−1	−0.5	0	0.5	1	1.5	2
H. takedai	M		20	4			1	6	3	5	5	1	2		
	F		4	3		1	4	2							
H. lichenatus	M		31	7					3	7	7	8	8	4	1
	F		2							1		1			
H. abei	M	2	22		1		4	6	12	1					
	F		30	4	3	8	16	5	1	1					

Figures indicate the number of specimens.

TABLE 4. Comparisons of number of vomerine teeth (mean±2SE) and of size (mean±2SE, in mm) and shape (median) of vomerine teeth series in the three forms of the *Hynobius lichenatus* group

Form	Sex	N	Vomerine teeth number	N	Vomerine teeth series width (VTW)	Vomerine teeth series length (VTL)	VTW/VTL
H. takedai	M	23	37.1±2.8 (28–54)	14	3.13±0.10 (2.85–3.58)	1.69±0.16 (1.07–2.17)	1.76 (1.46–2.66)
	F	7	35.6±4.7 (27–44)	7	2.88±0.11 (2.65–3.11)	1.69±0.20 (1.23–1.98)	1.68 (1.38–2.38)
H. lichenatus	M	37	34.2±2.4 (23–49)	16	3.43±0.12 (2.98–3.77)	1.78±0.12 (1.44–2.28)	1.97 (1.44–2.41)
	F	2	38.5 (36–41)		—	—	—
H. abei	M	24	36.3±1.9 (28–46)	20	2.76±0.16 (2.09–3.72)	1.97±0.10 (1.62–2.43)	1.37 (1.21–1.62)
	F	34	36.0±1.1 (31–43)	19	2.57±0.07 (2.28–3.03)	1.78±0.07 (1.35–2.10)	1.46 (1.25–1.69)

Figures in parenthesis indicate variation range.

comparison of the ratio of VTW to VTL ($U_{7,14}$=39, p>0.05).

Color in life — Dorsum uniformly dark brown or yellowish brown, females with well-scattered obscure spots; underside paler, usually with bluish white mottling in females. Females and non-breeding males often with small bluish white spots on sides and limbs.

Color in preservative — Dorsum uniformly dull brown with minute pale speckles; females often lighter with small darker spots scattered; undersurface grayish, with white speckles in some specimens, especially in females.

Measurements of the holotype (in mm) — Head width 12.8, snout to gular fold (head length) 15.6, head depth at posterior angle of jaw 7.2, eyelid length 3.6, anterior rim of orbit to snout 4.3, horizontal orbit diameter 2.7, interorbital distance 4.0, snout to insertion of forelimb 22.6, distance separating internal nares 3.6, distance separating external nares 3.7, projection of snout past mandible 1.1, snout to anterior angle of vent (SVL) 66.8, axilla to groin 33.8, anterior angle of vent to tip of tail (tail length, TAL) 49.3, tail width at base 10.2, tail height at base 10.1, tail height at middle 11.1, maximum tail height (MTAH) 11.6, axilla to tip of outstretched forelimb 16.8, groin to tip of outstretched hindlimb 20.0, width of vomerine teeth series 3.6, length of vomerine teeth series 2.5. The holotype has 78 upper jaw teeth, 37 vomerine teeth, 12 costal grooves between axilla and groin, 1.5 costal folds between adpressed limbs, and

5 digits on both hindlimbs.

Etymology — The specific name "*takedai*" is given after Mr. Toshio Takeda, former headmaster of Inoyama Elementary School, Hakui-shi, who first discovered the new form and has been continuously making efforts for its conservation.

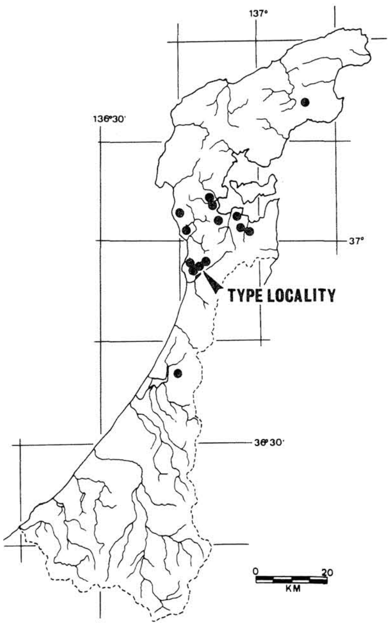

FIG. 2. Map of Ishikawa Prefecture showing locality records for *Hynobius takedai*.

Range — Known so far from lowland of Ishikawa Prefecture, on the Japan Sea side of the Chubu District, Central Japan (Fig. 2). Yanagida-mura: Gôroku; Nakajima-machi: Kawazaki, Shiotsu; Tatsuruhama-machi: Akakura-yama; Nanao-shi: Sohama-machi, Hosoguchi-machi; Kokubu-machi; Togi-machi: Fukuraminato; Shika-machi: Yaguradani; Hakui-shi: Shibagaki-machi, Taki-machi, Kashimaji-machi, Chiji-machi, Yanaida-machi; Tsubata-machi: Tsubata.

Morphometric comparisons — For comparison, 37 males and 2 females of *Hynobius lichenatus* from various localities of its range (Aomori, Akita, Niigata, Fukuṣhima, Tochigi, Gunma), and 24 males and 34 females of *H. abei* from Kyoto were examined. *H. takedai* was not different from *H. lichenatus* and *H. abei* in SVL in both sexes (Table 1), but males of *H. takedai* had smaller HL than males of the latter two species ($t=2.29$, dF$=59$, $0.02<p<0.05$ and $t=3.84$, dF$=46$, $0.001<p<0.002$, respectively). Further, males of *H. takedai* had shorter tail than *H. lichenatus* and lower tail than *H. abei*. These trends are quite evident when ratio of each dimension to SVL is compared (Table 2). Males of *H. takedai* had relatively short head and long trunk than males of *H. lichenatus*. At the same time, the tail was relatively short, but higher than in *H. lichenatus*. Males of *H. takedai* had relatively short and narrow head, long trunk and tail as compared with males of *H. abei*. Since only two females of *H. lichenatus* were available for this study, it was impossible to make statistical comparison with females of *H. takedai*. Females of *H. takedai* had relatively short and narrow head, low tail, and long trunk than females of *H. abei*. The three species did not differ in the number of costal grooves, and all had the modal number of 12 grooves (Table 3). The males of *H. lichenatus* had adpressed limbs usually overlapping about 0.5 costal folds (Table 3) and significantly differed from males of *H. takedai* in this character ($\chi^2=5.99$, dF$=2$, $p<0.001$). By contrast, in males of *H. abei*, adpressed limbs were more widely separated than in males of *H. takedai* ($\chi^2=17.35$, dF$=2$, $p<0.001$). In females, *H. takedai* did not differ from *H. abei* in the number of costal folds between adpressed limbs. ($\chi^2=0.80$, dF$=2$, $p>0.5$). The 5th toe is well developed in the majority of observed specimens in the three species. The number of vomerine teeth was not different between *H. takedai* and other two species, either, but the size of vomerine teeth series showed slight variation (Table 4): males of *H. takedai* had the teeth length similar to that in males of *H. lichenatus*, but had smaller width than in the latter ($t=3.55$, dF$=28$, $0.001<p<0.002$). Males of *H. takedai* had larger teeth width and smaller teeth length than males of *H. abei* ($t=3.47$, dF$=32$, $0.001<p<0.002$, and $t=3.21$, dF$=32$, $0.002<p<0.005$, respectively). Females of *H. takedai* also had significantly larger width than females of *H.*

abei ($t=4.09$, $dF=24$, $p<0.001$), but not different in length ($t=1.12$, $dF=24$, $p>0.2$). Thus, *H. takedai* was judged to have shallower vomerine teeth series than *H. abei* in both sexes as shown by significantly larger ratio of VTW to VTL ($U_{14,20}=12$, $p<0.05$, for males, and $U_{7,19}=21.5$, $p<0.05$, for females). No difference was found in this ratio between *H. takedai* and *H. lichenatus* in males ($U_{14,16}=78.5$, $p>0.05$).

Serum proteins and isozymes — To assess genetic diversity among three related species, we have examined, electrophoretically, the phenotypes of serum proteins, serum lactate dehydrogenase (LDH) and serum malate dehydrogenase (MDH) of 14 *H. takedai* from Akakurayama, Tatsuruhama-machi, 10 *H. lichenatus* from Niigata, and 33 *H. abei* from Kyoto. Electrophoresis was conducted in vertical polyacrylamide gels (7.5%) using Tris-glycin buffer (pH 8.3), for 2.5 hr at 1.5 mA DC/cm. Representative phenotypes resolved in this manner are shown in Figures 3–4. Inspections of the electrophoretic patterns of the three species revealed several significant findings: Moderately migrating serum protein fractions, which appeared as thin dark bands (arrows in Fig. 3), moved in *H. takedai* about the same rate as in some individuals of *lichenatus*, and were significantly in advance of the presumably homologous bands of *H. abei*. *H. takedai* was not polymorphic at the serum LDH–A locus and the band migrated identically with that of one electromorph of *H. lichenatus*, which showed polymorphism in this locus. Serum LDH–A of *H. abei* migrated significantly slower than in *H. takedai* (Fig. 4). Serum MDH bands showed less rapid electrophoretic migration in *H. takedai* than in *H. lichenatus*, which in turn exhibited significantly smaller mobility than in *H. abei* (Fig. 4).

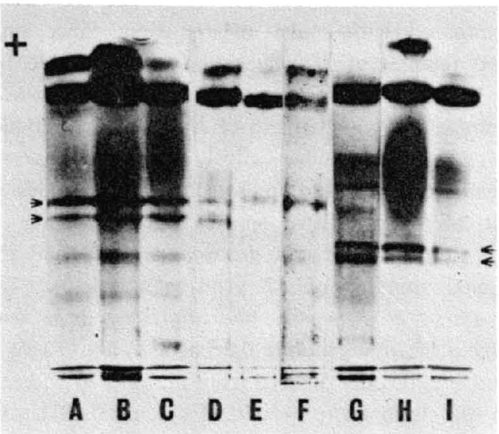

FIG. 3. Representative electrophoretic patterns of the serum proteins of *Hynobius takedai* (A–C), *H. lichenatus* (D–F) and *H. abei* (G–I). The arrows indicate moderately migrating fractions (see text).

Clutch size and egg sac — Another difference between *H. takedai* and the other two species is in the egg number and the appearance of egg sacs: *Hynobius takedai* overlapped *H. lichenatus* and *H. abei* in the variation range of clutch size (Table 5), but the mean (of 4 localities$=89.7\pm8.4$

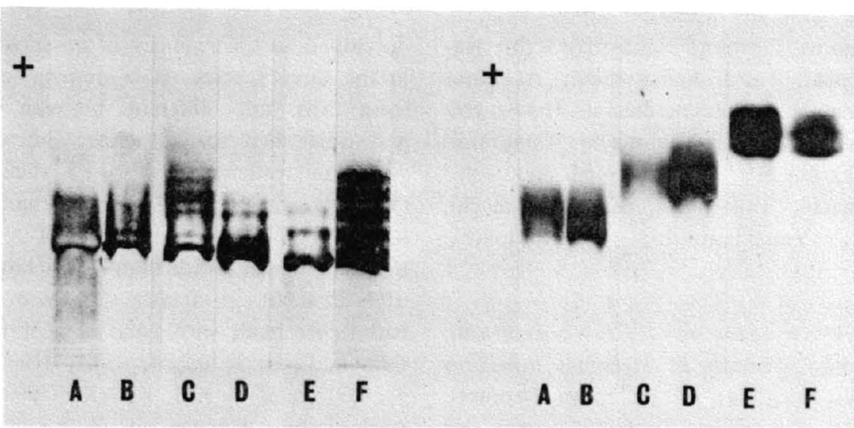

FIG. 4. Representative electrophoretic patterns of the serum LDH (left) and the serum MDH (right) of *Hynobius takedai* (A, B), *H. lichenatus* (C, D) and *H. abei* (E, F).

TABLE 5. Comparison of clutch size in the three forms of the *Hynobius lichenatus* group

Form	Locality		N	Range	Mean±2SE
H. takedai	Ishikawa	Chiji-machi	115	26–148	88.1± 3.7
		Yanaida-machi	47	40–108	67.7± 4.8
		Akakurayama	16	35–139	95.0±12.0
		Shiotsu	11	90–124	107.8± 8.5
H. lichenatus	Yamagata	Nezugaseki	10	36– 51	43.0± 3.4[1]
	Fukushima	Hinoemata	6	25– 37	31.0± 3.6
	Tochigi	Okushiobara	3	34– 53	46.0±12.0
	Gunma	Minakami	29	25– 58	41.7± 2.8
		Tanigawadake	35	24– 50	38.2± 2.0[2]
	Niigata	Tagami	60	18– 62	34.0[3]
		Kamo	92	33– 74	46.7± 1.6[4]
H. abei	Kyoto	Ohmiya-cho	10	43– 72	61.8± 5.4[1]
		Ohmiya-cho	4	52–109	87.5±32.3
		Mineyama-cho	5	45– 89	77.6±16.5
		Takeno-gun	7	26– 94	56.7±19.7

[1]Sato [2], [2]Iizuka [6], [3]Tôjio [7], values twice the original data reported for one egg sac (=1/2 clutch size), [4]Akiyama [8].

(2SE)) is evidently larger than those of the latter two species (mean of 7 localities=40.1±2.3, and of 4 localities=71.3±6.9, respectively). In addition, there are at most weak longitudinal wrinkles on the surface of egg sac in *H. takedai*, whereas the other two species have strong longitudinal striations which can easily be observed even with the naked eye.

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